修士論文要旨

The effects of EGCG on the growth of cancer cells lacking Rb gene

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Introduction

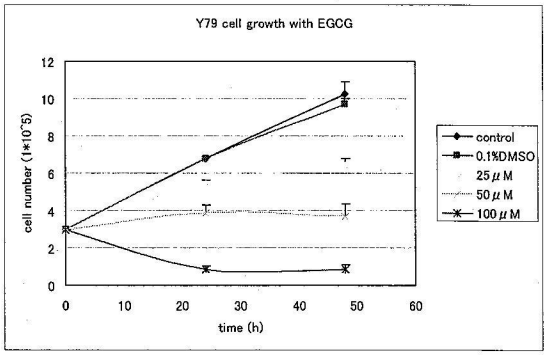
(-)Epigallocatechin-3-gallate (EGCG) inhibits the growth of cancer cells. Previous studies show that EGCG induces cell cycle arrest and/or apoptosis. It is considered that EGCG inhibits phosphorylation of retinoblastoma protein (Rb) and arrests cell cycle at G1 phase. It is also known that Rb, p107, and p130 are members of the "pocket proteins". Although not much is known about the exact roles of p107 and p130, it is thought that they substitute for Rb in Rb-deficient cells concerning cell growth inhibition. There are no studies that investigated the effects of EGCG on Rb-deficient cells. Therefore, I investigated whether EGCG inhibited the cell proliferation of Rb-deficient Y79 cells, and induced cell cycle arrest and/or apoptosis. Moreover, I examined the effects of EGCG on the expressions of p107 and p130 in Y79 cells.

Methods

Y79 cell growth and viability were examined using trypan blue exclusion method. EGCG-induced Y79 cell death was confirmed by measuring lactase dehydrogenase (LDH) activity, and by detection of DNA ladder in Y79 cells. The expressions of proteins such as p53, Bad, and capase3 which are involved in apoptosis were analyzed by western blotting. To elucidate whether EGCG treatment induced cell cycle arrest, cell cycle of EGCG-treated Y79 cells was examined using fluorescence-activated cell sorter (FACS). I also investigated the expressions of p107 and p130 using western blotting.

Results

I first examined the effects of EGCG on Y79 cell growth and viability. EGCG inhibited Y79 cell growth and viability in a dose-dependent manner. I next examined LDH activity and DNA ladder. LDH activity was increased, and genomic DNA of EGCG-treated Y79 cells was disrupted. Moreover, the expressions of p53 and Bad were increased by EGCG. The caspase3 precursor expression was decreased. These results suggested that EGCG induced Y79 apoptosis. FACS analysis revealed that EGCG did not arrest Y79 cell cycle. The expressions of p107 and phosphorylation forms of p130 did not change significantly.



Conclusion

The present study shows that EGCG induced apoptosis via inductions of p53, Bad, and caspase3, but did not induce cell cycle arrest of Rb-deficient Y79 cells. Though p107 and p130 were expressed in Y79 cells, both p107 and p130 did not substitute for the functions of Rb.